JAPANESE ENCEPHALITIS IN INDIA

INFORMATION DOCUMENT

NATIONAL INSTITUTE OF VIROLOGY

(Formerly Virus Research Centre)

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APPENDICES

This volume is dedicated to all those Medical and Public Health workers and the scientists who struggled indefatigably during the outbreaks of Japanese encephalitis in India.

This information document is compiled through the collaboration of many scientists of the NIV including Dr. N.P. Gupta, the former Director who initiated the project. A bibliography of the relevant work in India is appended.

K.M. PAVRI
Director
NATIONAL INSTITUTE OF VIROLOGY
(Formerly Virus Research Centre)

Pune 411 001
7th March, 1979

FOREWORD

Knowledge transmission is a key to understanding. The disease Japanese Encephalitis (JE), has caused considerable public anxiety in recent years. Caused by a virus and transmitted by mosquitoes, the occurrence of this disease highlights the interrelationship between man, his animals, birds and his environment. The symptoms are serious and dramatic. There is a high mortality. There is no specific treatment as yet that results in the elimination of the offending virus. Although the disease is known to be endemic in several parts of the country since the early '50s, its occurrence in recent years in an epidemic form in the southern and eastern parts of India poses a serious threat to public health.

Japanese encephalitis in India has several similarities with that occurring in Japan and other countries of eastern and south Asia; however, it presents many differences in its ecological and epidemiological features. The Council's National Institute of Virology in Pune, has been studying this disease extensively over the years, especially its causation and transmission. The knowledge base built by this Institute has been the main source of support to public health workers in the country in designing appropriate strategies in dealing with the recent outbreaks.

This booklet is an information document which summarises the present knowledge on various aspects of Japanese encephalitis including its causation, transmission, diagnosis, treatment and control measures. It depicts the work of the National Institute of Virology in an abridged form, useful for public health workers, clinicians working in hospitals and in the field and even the intelligent 'lay' public. The ICMR considers it as one of its major functions to disseminate knowledge on major public health problems of the

country from time to time. It is my hope that this document will serve a useful purpose and it is our intention to publish future editions as newer knowledge becomes available.

V. Ramalingaswami
Director-General
Indian Council of Medical Research

INTRODUCTION

The recent outbreaks of encephalitis in different parts of India have attracted considerable attention with regard to the aetiology in this country. Outbreaks of encephalitis or encephalopathies, especially in children, have not been uncommon in this country. Encephalitis can be caused by numerous viral and non-viral agents. Among the viruses, a number of arthropod-borne viruses are known to cause encephalitis. In India it is commonly caused by rabies virus. Viruses like herpes, poliomyelitis, other enteroviruses, mumps, chickenpox and measles are also known to cause sporadic cases of encephalitis. Infection of brain may also be caused by bacteria, fungi and protozoa like *Plasmodium falciparum*.

During the 1950's, outbreaks of encephalitis-like disease in young children were described from many places in India. Such outbreaks at Jamshedpur, Lucknow, Delhi, Nagpur and other places were investigated and are recorded. These investigations did not incriminate arboviruses as aetiological agents. Enteroviruses were suspected but only in an occasional case could they be possibly incriminated. The aetiology of these outbreaks remains undetermined. A monograph on this syndrome in children - named the acute encephalopathy syndrome - based on the investigations carried out jointly by several teams has been published by the Indian Council of Medical Research (ICMR). It has been suggested that the syndrome could be attributed to encephalopathy triggered off by exposure to excessive environmental temperature. Another suggestion worthy of consideration is that the disease resembles Reye's syndrome with the main lesion in the liver.

Encephalitis caused by Japanese encephalitis (JE) virus has been recorded in this country since 1955. Cases were first observed in Pondicherry and Vellore

in the mid-fifties. In a ten-year study period from 1955 to 1965, 52 proven cases of JE were reported from North Arcot and South Arcot districts of Tamil Nadu and the adjoining Chittoor district of Andhra Pradesh; 1955 and 1956 were known as epidemic years. In 1964, a small outbreak occurred in Madurai district of Tamil Nadu. In 1973, the disease broke out in Burdwan, Bankura and adjoining districts of West Bengal; the outbreak was extensively investigated by the National Institute of Virology (NIV), Pune, the School of Tropical Medicine, Calcutta, and the Medical College, Bankura. More than 340 cases were recorded in Bankura district of whom about 190 died. In 1975 and 1976, cases of encephalitis were recognized in these areas, but only to a limited extent.

In early 1978, a localized outbreak of JE was detected by the Bangalore Field Station of the NIV, in Kolar district of Karnataka State (number of cases affected: 72; number of deaths: 18). In February 1978, a major outbreak was detected in the district of Tirunelveli and the surrounding region (number affected: 299; number of deaths: 99). Later, during the summer-monsoon months, cases of JE were reported once again from Burdwan, Bankura and surrounding districts of West Bengal, as well as in Dhanbad district, Bihar. The NIV investigated hospitalised cases in Dhanbad and Asansol in August, September and October, 1978. At about the same time, an outbreak occurred in Dibrugarh, Assam, where some cases were also brought from Arunachal Pradesh. Evidence of JE was available through isolation of virus from CSF and/or brain tissues from Tirunelveli, Asansol and Dibrugarh. This was backed by serological data confirming the diagnosis of JE in a large proportion of cases.

An outbreak commencing in early October, 1978 occurred in Uttar Pradesh; Gorakhpur and the surrounding districts were the worst affected. Once again, JE virus was implicated as the aetiological agent. At about the same time, cases of encephalitis were also detected in the neighbouring districts of Bihar

State (see Appendix V,Table I). More recently,in 1979, outbreaks have been reported from several districts of West Bengal, Andhra Pradesh and Karnataka States.

JE IN OTHER COUNTRIES

Encephalitis due to JE virus infection occurs in Eastern Siberia, China, Korea, Japan,Taiwan, Malaysia, Thailand, Singapore and India. The disease in man shows a spring-summer seasonal incidence in temperate areas.

JE is maintained in nature by extra-human hosts. Man is an incidental host and plays no role in perpetuating the virus. The most important vector species in Japan and in most other areas is *Culex tritaeniorhynchus*. *Culex gelidus* is also thought to be an important vector in Malaysia and *Culex fuscocephalus* in Thailand.

Extensive studies on the epidemiology and ecology of JE virus were carried out from 1959 over a period of six years in and around Tokyo. *C. tritaeniorhynchus* was found to be the only mosquito species consistently infected and to be a very efficient vector. This is primarily a rural mosquito, breeds extensively in rice fields and preferentially bites large domestic animals but also feeds on birds and man. In Japan, the mosquito begins breeding in spring and reaches a population peak by the end of June. The important amplifying hosts of the virus were found to be black crowned night herons, egrets and pigs. Extensive pig breeding for meat in the Far-East has provided an important source for human epidemics. Pigs are attractive to the vector, circulate virus in high titres and have a rapid population turnover due to slaughtering. Man is not the preferred host for the vector and is not involved until a high density of infected mosquitoes is reached which, in Japan, occurs in late July. The disappearance of the virus begins in late September and presumably is due to the decline in the mosquito population.

In several outbreaks, a higher incidence of cases in children than in adults has been reported. This has been attributed to the progressive natural immunization of older age groups. There has also been evidence to suggest that children may be more liable to develop encephalitis.

JE IN INDIA

JE virus activity is not new to India. The earliest evidence of the prevalence of JE virus was obtained through serological studies carried out by the NIV (then the Virus Research Centre) in 1952. Neutralizing (N) antibodies to JE virus were found in Nagpur district of Maharashtra and Chingleput district of Tamil Nadu. In addition, sera from several scattered localities of Gujarat, Maharashtra, Andhra Pradesh and Tamil Nadu neutralized JE virus.

After this preliminary survey, more intensive efforts were made to look for JE virus activity in 1956. More than 4,000 sera were collected from 77 localities, in an area covering 9,700 square miles of southern India, by the NIV. The results showed that human infections with JE/WN viruses were extensive in Tamil Nadu and in parts of Karnataka State. Since then, the NIV has carried out extensive surveys among humans in different parts of India and the activity of flaviviruses including JE virus was found to be widespread throughout the country (MAP). Specific N antibodies to JE virus have been demonstrated in Tamil Nadu, Andhra Pradesh, West Bengal, Assam, Arunachal Pradesh and Rajasthan.

JE IN MAN

The disease has been recognized in India since 1955. Patients with acute central nervous system disease, seen between September and November 1955, at the Christian Medical College Hospital (CMCH), Vellore, North Arcot district, Tamil Nadu, were shown by workers of the NIV to have developed antibodies

against either JE virus or a closely related virus. To facilitate further study of the problem in southern India, a Field Station was set up at Vellore. In 1956, isolation of the virus of JE from wild caught mosquitoes definitely established its presence in India. However, the virus was not recovered from man until 1958; when three isolations were made from the brain tissues of cases of encephalitis. This served to confirm that JE virus was a cause of encephalitis in India.

During the 10-year period, 1955-1965, serological and/or virological evidence of infection with JE virus was detected in a total of 52 cases of encephalitis investigated in southern India by the NIV Field Station at Vellore. These cases represented only those which were seen at the CMCH, Vellore. There were undoubtedly many others which did not come to the attention of the hospital or health authorities.

In 1973, a large outbreak of JE occurred in Burdwan and Bankura districts of West Bengal during the months of May to October. Three virus isolations were made from specimens of brain. Serological evidence confirmed the aetiology of the disease. In 1974, the activity of JE virus among the healthy human population was detected through serological means. In 1975 and 1976, cases of encephalitis were recognized in West Bengal but only to a relatively limited extent.

During 1978, several outbreaks of JE occurred in different parts of India. From human cases, one isolate of virus from Tirunelveli, two isolates from Asansol and one from Dibrugarh were identified as JE virus at the NIV. Serological evidence to support the JE aetiology in these outbreaks was obtained. An isolation of JE virus was made at the KG Medical College, Lucknow, from brain tissue of a fatal case of encephalitis from Gorakhpur district. Serological evidence of recent JE virus infection was also shown by the NIV in sera collected from Gorakhpur and Basti districts. During 1979, outbreaks of Japanese ence-

phalitis occurred at Burdwan and Hooghly districts of West Bengal, Kolar and adjoining districts of Karnataka and Anantapur and Chittoor districts of Andhra Pradesh.

CLINICAL FEATURES

Published reports on the clinical and clinicopathological features of JE in India are available for Vellore, Tamil Nadu and Bankura, West Bengal. The Vellore report is based on a clinical and clinicopathological study of 16 cases, most of them serologically proved, in 1955. The Bankura reports are based on a clinical study of 143 cases, many of them serologically proven, and a clinicopathological study of 340 cases in 1973. This description of the clinical and clinicopathological features of JE is largely based on these reports.

In Vellore, all the cases were children below 15 years. Further, of a total of 52 proven JE cases studied at Vellore over a 10-year period from 1955 to 1965, only one patient was above 15 years. In contrast to this, the disease was seen in all age groups in the outbreaks that occurred in West Bengal, eastern Uttar Pradesh, Dhanbad area of Bihar and in Assam. However, even in these localities the incidence in children was higher than that in adults. Usually males were more affected than females and in most outbreaks, the male: female ratio was approximately between 1.5:1 and 2:1.

The disease was more commonly seen in people belonging to the lower socio-economic groups. In Bankura the incidence was higher in the scheduled castes and scheduled tribes who were closely associated with pigs.

The course of the disease can be conveniently divided into three stages - a prodromal stage preceeding signs of involvement of the central nervous system (CNS); an acute encephalitic stage marked by CNS signs and continuing fever; and a late stage marked by

recovery or the persistence of signs of irreversible neuron injury.

Prodromal stage

The essential features of this stage are general malaise, headache and fever. The onset of the illness is usually acute and is heralded with fever. Headache is often accompanied by vomiting.

The duration of the prodromal stage is usually between 1 and 6 days. However, it can be as short as <24 hours or as long as 14 days.

During the prodromal stage, no clinical diagnosis is possible as this picture is common to many infections.

Acute encephalitic stage

The predominant features of this stage are continuous fever, nuchal rigidity, convulsions, altered sensorium progressing in many cases to coma, focal CNS signs, polymorphonuclear leucocytosis in the peripheral blood and CSF changes marked by pleocytosis with a normal or raised sugar and slightly raised protein.

Fever is usually high and the temperature varies from 100°F to 107°F. The duration of fever is variable. In the Vellore cases, it varied from 3 days to several weeks. In Bankura, it generally continued for 7 to 10 days and relative bradycardia was a common feature.

Nuchal rigidity is a common feature. It was present in 82 per cent of cases seen at Bankura and in 12 out of 16 cases studied at Vellore. Neck stiffness was a more conspicuous feature than positive Kernig's sign.

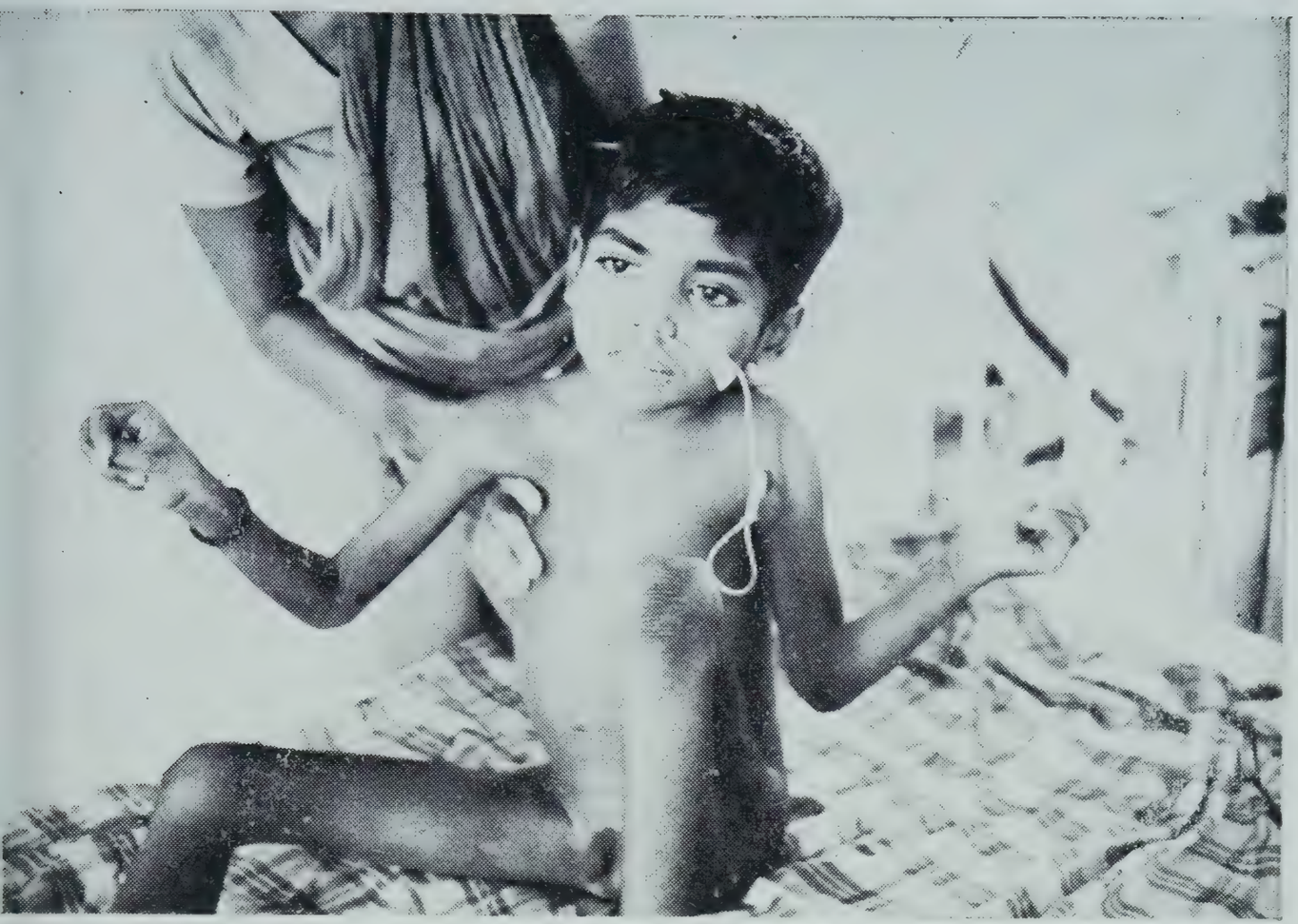
Convulsions are a common feature, especially in children. At Vellore, convulsions occurred in all except two of the 16 cases and were mostly generalised. In the great majority the fits took the form of grand mal epilepsy with repeated clonic movements often of considerable violence. In a few cases tonic fits occurred throwing the child into opisthotonus with retracted head, extended legs and arms flexed at the elbow.

In Bankura, where for the first time in India a number of patients were adolescents and adults, convulsions were seen in about half the patients and were mostly focal in type.

Altered sensorium of varying degree occurred in all cases. In Bankura, this followed 2 to 3 days after onset and was characterized by clouding of consciousness, confusion, delirium, disorientation, stupor and finally, in many cases, coma. In Vellore, all cases progressed to coma and the duration of the coma varied greatly, from a few days to several weeks. In all cases, at some time, coma was moderately deep so that really firm supra-orbital pressure produced only a slow contortion of the face and hard pinching of the tendo Achilles a feeble and incomplete attempt at withdrawal of the foot.

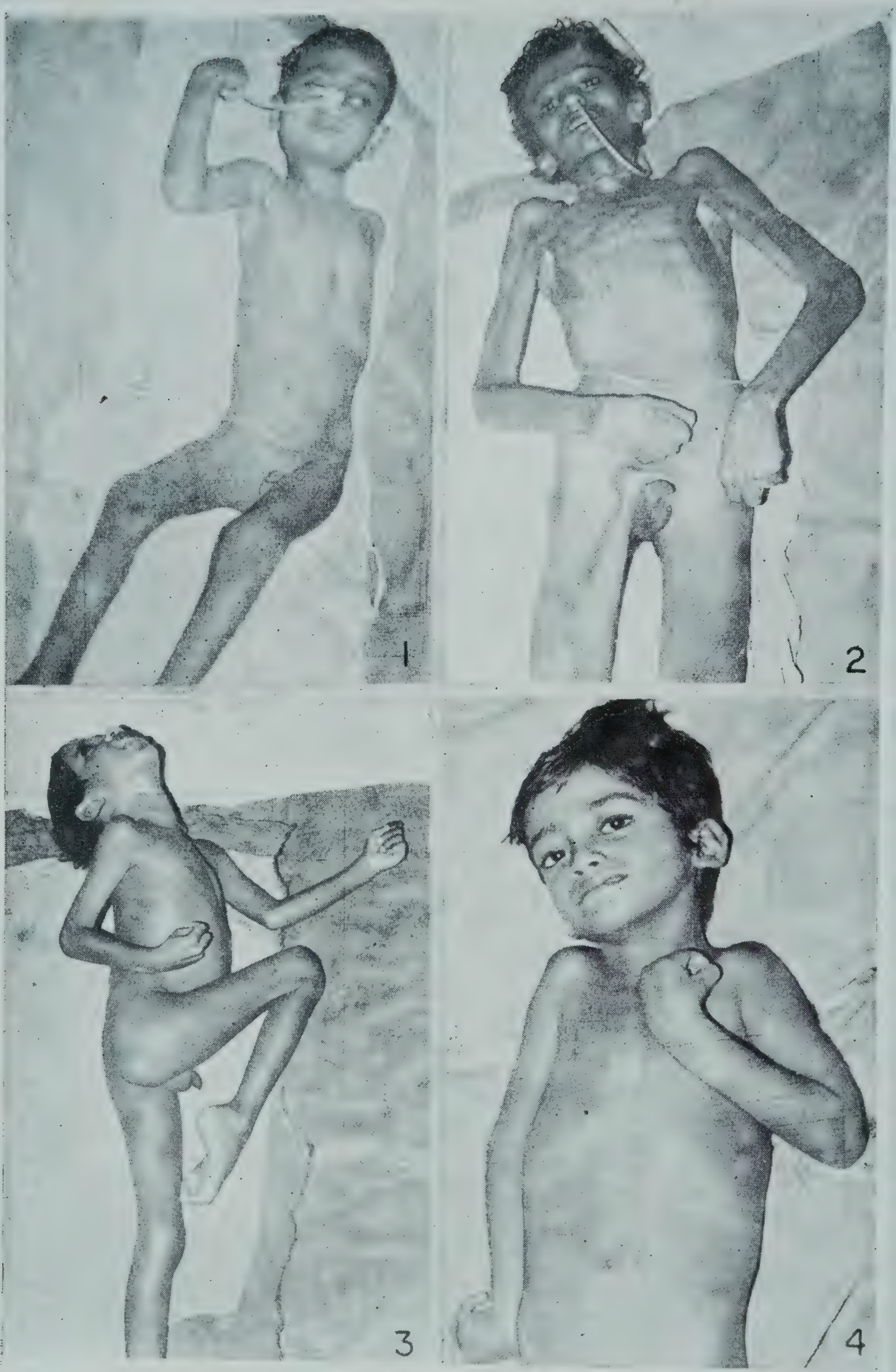
Focal neurological signs were detected in both Bankura and Vellore patients. They consisted of some form of paralysis and involuntary movements such as choreo-athetosis, constant shaking of the head from side to side smacking of the lips, constant flailing of limbs and rolling of the trunk.

Neurological signs were variable. In Bankura, pupils were found to be of variable size but generally equal on both sides. Light reflex was preserved. There was no ophthalmoplegia or nystagmus. Ophthalmoscopic examination showed little abnormality. In Vellore, papilloedema was present in 6 of the 16 cases.



A Japanese encephalitis patient during an epidemic in Tirunelveli district, Tamil Nadu, 1978

Paddy fields with standing water are the main breeding places for mosquitoes transmitting Japanese encephalitis



Encephalitis patients during the 1979 epidemic in Kolar district, Karnataka State

1. Acute stage, patient expired. 2. Emaciation, patient in recovery phase.
3. Dystonic posture with neck retraction; 4. Dystonic posture of limbs and a vacant look.

(*Courtesy* of Sh. H R. Ramachandraiah, Bangalore Medical College, Bangalore).

In Vellore, an extensor plantar response was found in some children in the early stages but in no child did this persist. Tendon reflexes were always present, though at times reduced in the limbs affected by paresis. In children in whom the acute encephalitic stage lasted for many weeks, the affected limbs showed marked spasticity with exaggerated tendon reflexes, clonus and a tendency to develop contractures. In Bankura, the jerks were more often sluggish than brisk, but generally equal on both sides. The plantar response was extremely variable and often dissimilar on the two sides.

Other systems: Apart from CNS signs, there were few significant findings. In the Bankura study some non-specific electrocardiographic changes were found in a few patients. In the Vellore study, 2 of the 16 patients studied showed evidence of myocardial strain in the form of apical gallop rhythm. This was present at the height of fever, and was observed for 1 and 2 days, respectively. Neither showed any significant anaemia or evidence of pre-existing heart disease.

Blood leucocyte count: A majority of cases both at Vellore and Bankura showed moderate to high polymorph leucocytosis. The total counts ranged between 10,900 and 34,800 for the Vellore cases, the neutrophils forming 51 to 90 per cent. In the Bankura cases, the majority had total counts between 10,000 and 25,000, with a mean total leucocyte count of 12,200 ± 4,775; the mean neutrophil count was 69.23 per cent of the total.

Cerebrospinal fluid: At Vellore, there was pleocytosis in the CSF in all cases in the acute stage. The observed cell counts ranged from 10 to 980 per cmm; it was above 50 per cmm in 10 cases, but above 200 per cmm in only 3. The differential count followed no uniform pattern, some cases showing a predominance of lymphocytes, others of neutrophils. The protein was raised in 9 of the 16 cases, but never above 90 mg

per cent. CSF sugar was raised in some cases. The chlorides were within normal limits.

At Bankura, of 210 cases, 127 had counts ranging between 6 and >100. The mean cell count was 27.08. The cells were mostly mononuclear. The mean concentration of protein in the CSF (40.6 mg per cent) was slightly higher than the average protein content of a normal CSF sample. The mean sugar content of the CSF (77.9 mg per cent) was also slightly higher than the normal average, but this may be attributed to the effect of intravenous infusion of dextrose solution before collection of the CSF samples. The mean value of CSF chloride (630.25 mg per cent ± 21.9 mg per cent) was lower than the normal average.

Electroencephalogram: EEGs were recorded in 7 Vellore cases during the acute encephalitic stage and were found to be grossly abnormal. The outstanding features were dimunition of electrical activity, dysrhythmia and slowing. These changes tended to improve during recovery.

The clinical picture could be summed up as follows:-

Symptoms/Signs	Frequency
Fever	++++
Headache	+++
Altered sensorium (including coma)	++++
Convulsions	+++
Abnormal movements	++
Neck rigidity	+++
Kernig's sign	++
Blood leucocytosis	+++
CSF pleocytosis	+++
CSF increased protein	++

Late stage

This stage begins when active inflammation is at an end *i.e.* when temperature and ESR are completely normal and neurological signs stationary or tending to improve.

When the encephalitic stage was short, recovery occurred rapidly and the patient became normal within 2 to 4 weeks of the onset of illness. When the encephalitic stage was prolonged, recovery was correspondingly slower. Most of the patients had a prolonged convalescence and residual neurological deficits were not uncommon.

The case-fatality rate at Bankura was 58.7 per cent; mortality was highest among those above 40 years of age. Most of the patients who died developed terminal pulmonary oedema. The average period between the onset of illness and death was 9.1 days. At Vellore, of the 52 proven cases studied between 1955 and 1965, approximately 10 per cent died. The actual case-fatality rate may, however, have been considerably greater, for adequate samples for laboratory diagnosis were often not available from children who succumbed soon after entering the hospital. In other outbreaks in India the case-fatality rate has varied between 25 and 45 per cent.

TREATMENT AND MANAGEMENT OF PATIENTS

At present, there is no specific treatment. Treatment is essentially symptomatic and consists of control of hyperpyrexia and convulsions, reduction of increased intracranial pressure, and careful management and nursing, the latter being of the utmost importance. Care should be taken to maintain unobstructed airways and adequate nutrition. While comatose patients should be nursed in the semiprone position with the foot end of the cot raised, the position should be changed, as far as possible, every two hours to avoid bedsores, and careful attention should be given to

pressure points. Unconscious patients should be fed through an intragastric tube.

Distention of the bladder must be anticipated and controlled by an in-dwelling catheter.

Control of pyrexia

This can be accomplished by antipyretics, cold sponging or ice packs.

Control of convulsions

In children, phenobarbital, 5 to 8 mg/kg/24 hours is given in an effort to prevent convulsions. If frequent or sustained convulsions appear, it may then be necessary to give diazepam intravenously (0.1 to 0.2 mg/kg).

In adults, seizures may be aborted with a slowly administered intravenous bolus of diazepam (10 mg). To prevent further seizures, a loading dose of 200 to 600 mg of phenobarbital, 100 to 200 mg being given intravenously at a rate not exceeding 25 mg per minute and the remainder intramuscularly in divided doses, should be administered. A maintenance daily dose of 200 to 300 mg of intramuscular phenobarbital should prove adequate.

Reduction of cerebral oedema and increased intracranial pressure

Reduction of cerebral oedema and increased intracranial pressure is of prime importance since avoidance of consequent anoxia will preserve those cells that are not irreversibly damaged. A number of methods are proposed to minimize cerebral oedema and reduce elevated intracranial pressure:

1. In children, dexamethasone, 0.25 to 0.5 mg/kg/24 hours, is given intramuscularly. In adults, the dose is 10 mg intravenously initially, and then 4 mg every

4 to 6 hours. These large doses should be reduced gradually after a few days if recovery or improvement is evident. The possible benefit derived from corticosteroids when cerebral oedema is clinically significant appears to outweigh their potential for immunodepression.

2. In children, mannitol is given intravenously in a dose of 1.5 to 2.0 gm/kg as a 20 per cent solution over a two-hour period. This may be repeated every 8 to 12 hours. For an adult, 500 ml of a 20 per cent mannitol solution can be administered over half to one hour (7.5 to 10 ml/kg). The repeated use of osmotic diuretics is decreasingly effective and rebound increases in the intracranial pressure can occur. Therefore, osmotic diuresis can be considered only as a temporary measure and should be followed with more definitive therapy such as surgical decompression or corticosteroids.

3. Glycerol, by nasogastric tube using 1.5 mg/kg diluted with twice the volume of orange juice can be administered. This is non-toxic and may be repeated every 8 hours for an extended period of time.

4. Artificial hypothermia may be tried and should maintain a body temperature of approximately 31.5°C (89°F) for 3 to 4 days.

5. Early surgical decompression may be useful both to alleviate increasing intracranial pressure and to provide a definite diagnosis by brain biopsy, where facilities exist.

Fluid and electrolyte replacement

Patients when first seen may be dehydrated due to fever, vomiting and insufficient intake of fluids. Intravenous infusion of 5 per cent glucose in normal saline for adults or in half concentration saline for children may be used. Excessive rehydration should be avoided because of the danger of pulmonary oedema.

Prevention of secondary infections

Parenteral administration of antibiotics, preferably broad-spectrum antibiotics, such as ampicillin, should be used to prevent respiratory infection and urinary tract infection resulting from catheterization.

Follow-up therapy

Physiotherapy, occupational therapy and corrective surgery should be utilized as necessary. In children, transient emotional and behavioural aberrations are common. Children return to normal more quickly in a permissive rather than a too restrictive atmosphere; parents should be advised about this.

Supportive and rehabilitative treatment are very important during the follow-up period.

Rehabilitation, most of the time, does not require sophisticated hospital treatment. Advice, based on physiological principles can be usefully provided to patients in primary health centres. Physicians should be aware that this is a very important part of the treatment leading to complete recovery of patients.

SYNOPSIS OF TREATMENT*

1. Control of pyrexia : Antipyretics, cold sponging or ice packs.

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* Further details of treatment and management of patients are available in the following:

Steigman, A.J. Encephalitis. In: *Nelson's Textbook of Paediatrics* edited by V.C. Vaughan, R.J. McKay and W.E. Nelson (consulting editor), 10th Edition (Asian Edition), Tokyo, Tgaku Shoin Ltd. pp. 723-729, 1975.

Wolinsky, J.S. Viral Meningoencephalitis. In: *Current Therapy* edited by H.F. Conn, Philadelphia, W.B. Saunders Company, pp. 705-707, 1978.

2. Control of convulsions :

Children:

Phenobarbital 5 to 8 mg/kg/24 hours (to prevent convulsion).

Diazepam 0.1 to 0.2 mg/kg, *iv* (if convulsions are sustained).

Adults:

Diazepam 10 mg *iv* to abort seizures).

Phenobarbital 200 to 600 mg (loading dose), 100 to 200 mg given *iv* initially, followed by the rest *im* in divided doses. Maintenance dose 200 to 300 mg *im* per day to prevent convulsions).

3. Reduction of cerebral oedema and increased intracranial pressure :

Children:

Dexamethasone, 0.25 to 0.5 mg/kg/24 hours *im*.
Mannitol (20 per cent solution) 1.5 to 2 gm/kg over a two-hour period.

Glycerol 1.5 mg/kg diluted with twice the volume of orange juice.

Adults:

Dexamethasone 10 mg stat *iv*, followed by 4 mg every 4 to 6 hours.

Mannitol (20 per cent solution) 500 ml *iv*, administered over half to one hour.

Both children and adults:

Artificial hypothermia. Early surgical decompression, where facilities exist.

EPIDEMIOLOGICAL FEATURES

As determined by serological surveys carried out by the NIV between 1955 and 1972, JE virus infection is widespread in India and is particularly high in the southern states of Andhra Pradesh, Tamil Nadu and parts of Karnataka. It was also found to be prevalent in Orissa, Assam and the lower elevations of Arunachal Pradesh as well as in Rajasthan. Until 1973, the disease, however, was not commonly recognized and the occurrence was restricted to southern India. The ratio of inapparent human infections to clinically apparent disease is high for JE. In Japan, it was found to be between 500 and 1000 inapparent infections for every case of JE. In India, it is probably similar to that in Japan as judged by the high proportion of the population possessing antibodies to JE virus in the endemic areas and the relatively low incidence of the disease. Control studies have, however, not been carried out.

The disease in southern India almost exclusively affected children below 15 years, while in West Bengal all age groups have been affected. This would appear to indicate that the virus in this area has been newly introduced into a relatively non-immune population. In most of the epidemics the incidence has been higher in males. In Bankura district, lower socio-economic groups had a higher incidence.

Another feature of JE is the scattered pattern of incidence. On an average, 1 to 1.5 cases occurred per village and there was not more than one case in a household.

The seasonal incidence of JE in humans has not been the same in different parts of India. In southern India, the illness occurred mainly during the later half of the year, coinciding with the rainy season and period of high mosquito prevalence. Tamil Nadu receives, in addition to the south-west summer monsoon, rainfall due to the north-east winter monsoon. The peak incidence of JE was in October to December. The exception was the outbreak in Tirunelveli and surrounding districts in 1977-78 which commenced in November and lasted till April with the peak in February and March.

In eastern India the disease occurred between May and October. In Bankura this was shown to be related to the summer monsoon. In Assam and Uttar Pradesh the recent outbreaks have occurred between September and December. In Uttar Pradesh in 1978, the outbreak followed extensive floods. This could possibly be due to an extensive increase in the vector mosquito density by an increase in breeding sites. However, it was not possible to prove this association by controlled studies.

In 1971, prospective studies were commenced by the NIV in Andhra Pradesh. The aim of these studies was (a) to study the epidemiology of JE in the richly irrigated and fertile Krishna-Godavari delta, and (b) to compare the activity of JE virus in the above mentioned area with the activity in an area located in Khammam district - which was relatively not well irrigated but which was soon likely to come under irrigation from the left bank canal of the Nagarjuna Sagar project - both before and after irrigation.

Several methods were utilized to obtain data on the activity of JE virus, *viz.*, serological surveys

among humans, pigs and birds; mosquito collections and attempts to isolate virus from mosquitoes; sentinel animal studies employing young pigs and chicks; and a survey of records of hospitals and primary health centres to estimate the incidence of encephalitis.

Valuable base-line data were obtained between 1971 and 1975 when the study was temporarily suspended. The JE/WN complex of viruses were found to be prevalent in all three districts. However, the prevalence of JE virus appears to be somewhat lower in Khammam district. A significant proportion of birds of the family Ardeidae were found to possess antibodies to JE virus.

The study will be resumed after canal irrigation is well established in Khammam district.

DIAGNOSIS OF JE

A rapid specific diagnosis of sporadic disease with JE virus is not yet possible. In an epidemic situation, the symptomatology, detailed clinical examination, examination of the CSF and the epidemiological picture can help to arrive at a diagnosis. Under these circumstances, the diagnosis of the aetiology of the epidemic assumes importance. This can be achieved by:

1. Virus isolation from brain biopsy/necropsy specimens to be transported in transport medium (vide Appendix II) or CSF or very rarely from the blood of the patient.

2. Demonstration of virus antigen in the brain of patient.

3. Demonstration of production of specific antibodies in the patient's sera - either by seroconversion or rise in antibody titre against JE virus.

Laboratory tests for diagnosis of JE

The laboratory diagnosis of JE virus can be made by the demonstration or isolation of the virus in the test specimen and by identification of the isolate by serological methods.

In the absence of isolation of the virus, serodiagnosis may be of help. The methods for collection, storage and transport of specimens are described in Appendix I.

Virus detection

Demonstration, isolation and identification of the virus:

1. Demonstration of the virus/viral antigen in the autopsied brain tissue by fluorescent antibody technique (direct and indirect method).

2. Isolation of the virus from autopsied brain tissue, CSF and occasionally from peripheral blood collected during the very early phase of illness. Isolation is usually carried out by intracerebral inoculation of the specimen in infant mice or by infecting cell cultures (Vero, primary MKTC, hamster kidney

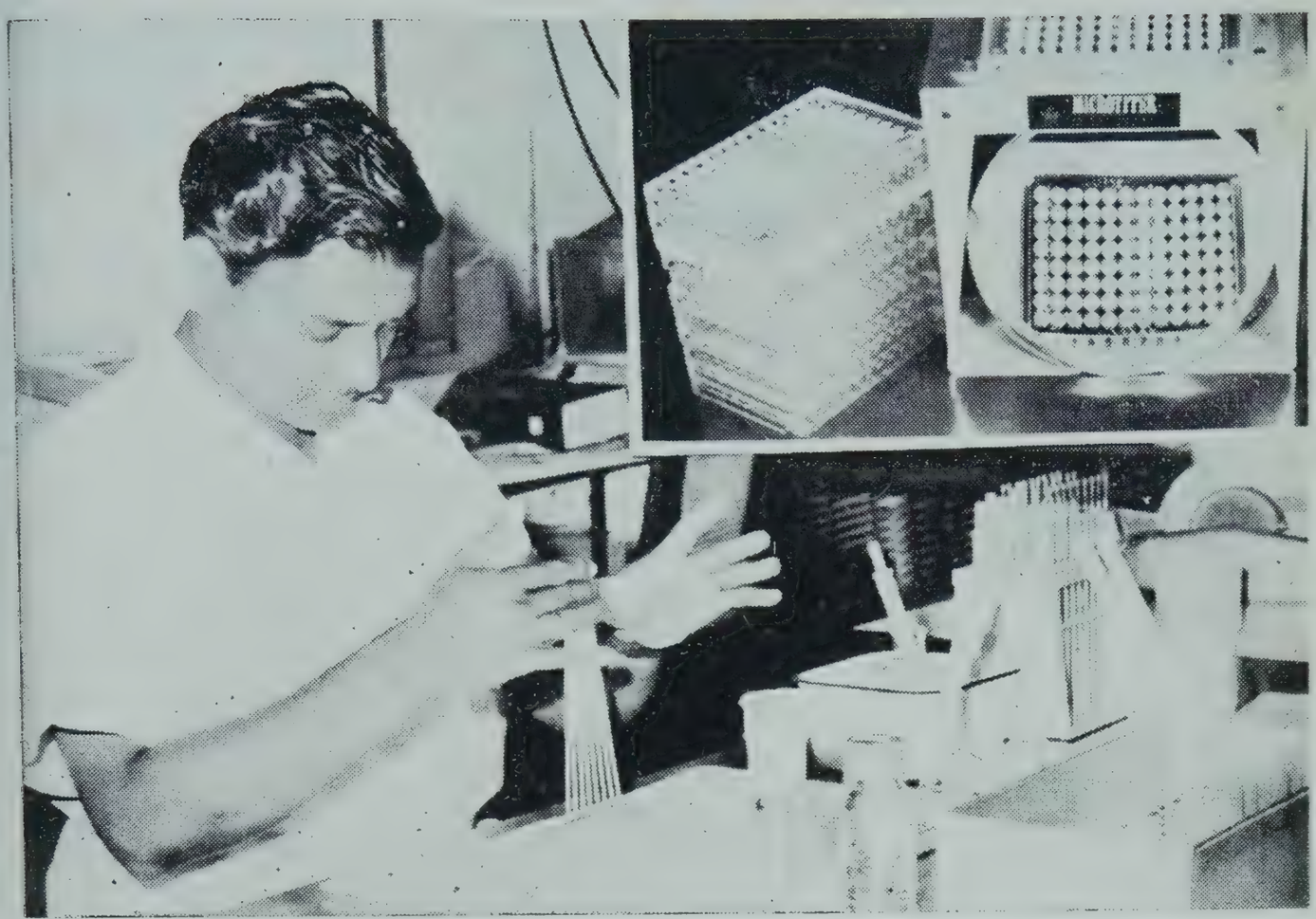
3. Identification of the agent is done by serological tests. Usually by the complement fixation (CF) test using antisera to JE, WN, and dengue-2 viruses. Since JE and WN viruses show cross-reactions, specific identification can be made using kinetic CF test at 0 hour (immediate), 3 hours or 18 hours of incubation before adding the haemolytic system. Alternatively, JE/WN monospecific antisera obtained by absorption of cross-reacting antibodies are used. Agar gel diffusion (AGD) tests can also be used for identification of the agent. However, in such cases, antigen has to be prepared from brain tissue.

Serological tests

In the absence of isolation, diagnosis can also be based on the rise of antibody titre in the paired sera collected at an interval of 15-20 days.

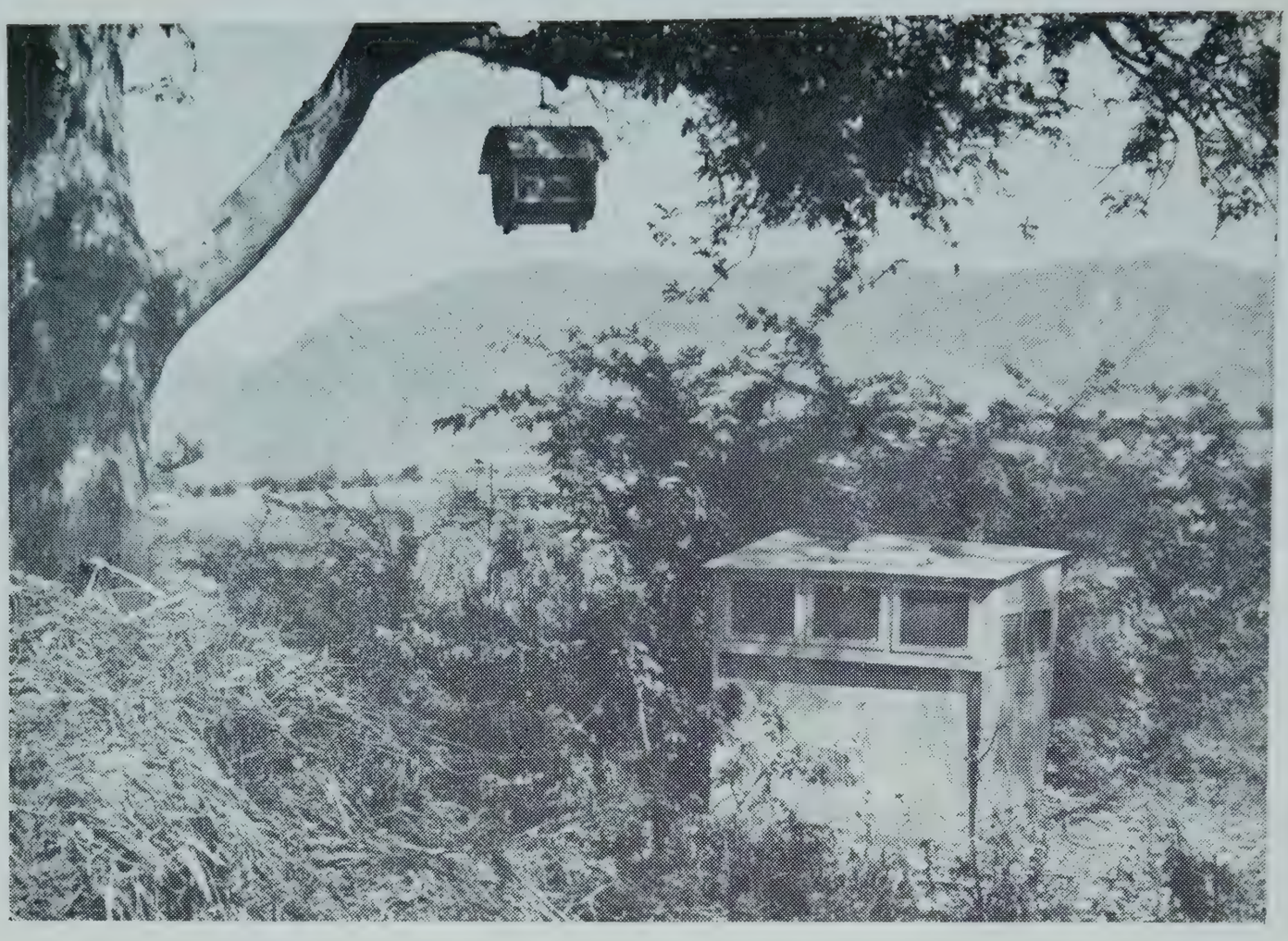
Serological response could be primary or secondry. A primary response is considered when there is no previous flavivirus infection and there is monotypic rise of antibodies to JE virus only. However, quite often, primary monotypic response is not obtained and some degree of cross-reaction with WN virus may be obtained even in primary response. In this case, serological diagnosis is not difficult as the titre of the cross-reacting antibodies is lower by at least fourfold. Usually haemagglutination/complement fixation tests are employed. There is rise in titres of HI as well as CF antibodies. Single phase convalescent sera should show high titre of HI and CF antibodies (HI $\geq$ 1:80 and CF $\geq$ 1:32). However, in such cases, it is essential to determine the presence of early IgM antibodies. This is done by determining 2-ME sensitive IgM antibodies in HI tests. If a serum sample shows a difference of $\geq$ 3 tubes of 2-ME sensitive antibodies as evidenced by a drop in 2-ME treated sera, it may be considered as evidence of recent infection.

Secondary response: Where there is previous infection of related flaviviruses there is rise of antibody titre to all the antigenically related viruses such as WN, dengue, *etc*. Such cases should be carefully scrutinized before making any definite diagnosis. Usually, there is a fourfold or more increase in the titre of JE antibodies in comparison to other viruses. Moreover, JE antibodies show ME sensitivity in some cases, but in sera having high CF and HI antibodies and when samples are collected late, *i.e*. after 20 days or more, ME sensitive antibodies may not be detected.



Performance of a serological test to detect antibody to Japanese encephalitis in sera from different sources

Blood being collected from a suspected case of Japanese encephalitis for etiological studies



The chick-baited trap (suspended from the tree) and the pig-baited trap (on the ground) being used during ecological studies on the vectors of Japanese encephalitis virus

Mosquitoes being fed on chicks infected with Japanese encephalitis virus during experimental transmission studies

Neutralization (N) tests: This is a specialized test which gives a more definite diagnosis. The tests are carried out with paired sera either by *ip* or *ic* routes in mice or in cell cultures.

Agar gel diffusion (AGD) tests: AGD tests being specific, JE antigen can be made use of for detecting JE specific antibodies. Only those sera containing high titres of antibodies can be satisfactorily tested.

Virus isolation from mosquitoes

Different species of mosquitoes act as vectors of JE in different parts of the world. Isolation of virus from the vector mosquitoes is an integral part of epidemiological studies on JE in an area of affliction.

The methods of collection and transportation of mosquitoes have been described elsewhere (Appendix III). For virus isolation the following procedure is adopted:

Pools of mosquitoes are incubated at room temperature for 30 minutes by putting them in vials containing saline with penicillin 4000 units/ml, streptomycin 2 mg/ml and kanamycin 2 mg/ml. The mosquitoes tend to float in saline and, therefore, the vials are intermittently shaken. Following incubation, the mosquitoes are taken out from saline, ground with a mortar and pestle and a suspension made in phosphate saline containing 0.75 per cent bovalbumin (BAPS) with penicillin 1000 units/ml and streptomycin 1 mg/ml. For pools up to 50 mosquitoes, 1.5 ml of BAPS is used. For larger pools 2 to 2.5 ml may be used. Very large size pools of mosquitoes are not convenient for processing. The mosquito suspension is centrifuged at 4°C at 12100 g for one hour, following which the supernatant fluid is carefully taken out by means of a capillary pipette. The clear supernatant fluid is inoculated into mice or cell cultures.

NATURAL HISTORY OF JE

Most of the arboviral infections are zoonotic diseases having their natural cycle in wild or domestic vertebrates and haematophagous arthropods. They are generally maintained in enzootic forms and appear as focal outbreaks under specific ecological conditions. JE virus infection is no exception.

Arthropod vectors

During the studies in different parts of India, 29 strains of JE virus have been isolated from mosquitoes. Of these, 22 were isolated from peninsular India and 7 from West Bengal. In the earlier part of these studies, the taxonomic status of the species belonging to the *Culex vishnui* complex was not clear and all the species under the group were clubbed as the *"Culex vishnui"* group and the isolations made in 1956 were attributed to the *"C. vishnui"* group. Subsequently, the three species belonging to the *C. vishnui* complex, *viz.*, *Culex tritaeniorhynchus*, *C. pseudovishnui* and *C. vishnui* were distinguished on taxonomical grounds and the isolations made thereafter were attributed to each species.

Of the 13 isolations made after 1956, eight were from *C. tritaeniorhynchus*, three from *C. vishnui* and two from *C. whitmorei*. The ecological studies, particularly on the habitats, the relative population density, biting habits, host predilection and on the vector potentials incriminate *C. tritaeniorhynchus* as the major vector in peninsular as well as in the other parts of India. *C. vishnui (C. annulus)* which is closely related to *C. tritaeniorhynchus* is considered to be the main vector in Taiwan. In India *C. vishnui* may be another important vector as recently JE has been isolated from this species from West Bengal and Kolar district of Karnataka. These mosquitoes as compared to *C. tritaeniorhynchus* have been shown to be attracted more to children, birds and pigs. In Thailand, *Culex fuscocephalus* and in Malaysia, *Culex*

gelidus are also known to be vectors, but apparently of lesser importance. However, there have been no isolations of JE from these species in India. The two isolations from *C. whitmorei* from North Arcot district and Krishna district incriminates it as a possible vector.

During the 1973 epidemic of JE in the Asansol area in West Bengal, three strains of JE virus were isolated - one each from *C. vishnui*, *Anopheles barbirostris* and *A. "hyrcanus"** at the School of Tropical Medicine, Calcutta. During the follow up studies in 1974-75 in Bankura district, 4 more strains were isolated. Two of them were from *A. "hyrcanus"* and one each from *Culex bitaeniorhynchus* and *Culex epidesmus*. Subsequently, 2 strains, one each from *C. vishnui* and *A. subpictus* were isolated from Kolar district. The five isolations from Anophelines have added new dimension and complexity to the natural history of the disease. There has been a report of virus isolation as well as laboratory transmission of virus, in *Anopheles sinensis*, a species closely related to *A. nigerrimus*, in Japan. Transmission of virus by *C. tritaeniorhynchus* and isolation and transmission in *C. pipiens pallens* have also been reported. Recent laboratory experiments undertaken at the NIV demonstrated that *Anopheles tessellatus* is capable of transmitting the virus. However, *A. "hyrcanus"* collected around Pune did not transmit it, although it retained the virus for nine days. The vector potential of *A. "hyrcanus"* needs confirmation, particularly the strain found in West Bengal.

The isolation of virus from *C. bitaeniorhynchus* is noteworthy. Laboratory studies have determined its high vector potential. In Bankura district, the species was found in all types of collections but more frequently found in chicken baited traps, as well as

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* *A. hyrcanus* group in India is represented by six species. Of these, *A. nigerrimus* and *A. peditaeniatus* are widely distributed.

human biting collections. The aviphilic and anthropophilic behaviour of the mosquito incriminates it in transmission from bird to bird as well as to man. *C. epidesmus*, which yielded one isolation, is found in northern India. During the year-round studies at Bankura district, this species was found in significant numbers only from July to November and was present in all types of collections. Until more information is available on its habitat, habit and vector potential, it is difficult to evaluate its role in the natural history of the virus.

The available information obviously incriminates mosquito species belonging to *C. vishnui* group. Other species might be acting as complementary vectors. The transmission and maintenance system of JE appears to be complex and may vary from area to area depending upon many ecological factors than mosquitoes alone. With the available information it is not possible to assess the relative role of these factors in the epidemiology of the disease.

The following observations on the bionomics of some vector species might be added. The two main approaches employed at Vellore to study the biting habits of vectors were: (i) the host predilection for various animal baits and (ii) the analysis of mosquito blood meals by precipitin tests to determine the hosts on which they had fed. These studies showed that members of the *C. vishnui* complex are more attracted to cattle than man. Of the three species under the group, *C. tritaeniorhynchus* is least attracted to man. They are less attracted to birds than cattle and again *C. tritaeniorhynchus* is the least attracted. The number of pig-feeds as detected by precipitin tests was low with all the three species. However, they fed well when exposed to pigs in pig-baited traps. The relatively low level of pig-feeds detected in wild-caught mosquitoes probably reflects the relatively low pig population as compared to human and cattle.

Vertebrate hosts

Birds: The birds belonging to the family Ardeidae have been incriminated both in the maintenance and dissemination of JE virus in Japan. In India too, birds have been suspected but until recently no full scale investigations were conducted to assess their role.

The first of a series of investigations was conducted between 1955 and 1965 at Vellore and 1396 birds, representing 71 species, were processed for virus isolation. No strain of JE virus was isolated. Sera from 410 birds, 71 of which belonged to the family Ardeidae, were screened for N antibodies against the virus and no incriminating serological evidence was obtained.

During the subsequent studies carried out in Andhra Pradesh, sera from 286 *Ardeola grayii* (pond heron) and 229 *Bubulcus ibis* (cattle egret), both belonging to the family Ardeidae, were screened for N antibodies against JE and 110 (38.5 per cent) of the former and 85 (37.0 per cent) of the latter showed the presence of antibodies. Experimental viraemia and bird-to-bird transmission of the virus through *C. tritaeniorhynchus* mosquitoes showed that pond herons and cattle egrets develop viraemia in sufficiently high titres to infect mosquitoes and that mosquitoes fed on them effectively transmit the virus.

In West Bengal, in many areas, ducks were found in large numbers living in close association with man under conditions thought to be favourable for virus transmission. A study on experimental viraemia and duck-to-duck transmission through the mosquitoes showed that ducks developed adequate levels of viraemia and the mosquitoes fed on them get infected and effectively transmit the virus. However, when 104 bird sera were tested, only 8 showed the presence of antibodies. No information is available on the immunity status of ducks in the Bankura area. In the Andhra

area only 5.7 per cent of ducks (3 out of 53) possessed N antibodies to JE virus.

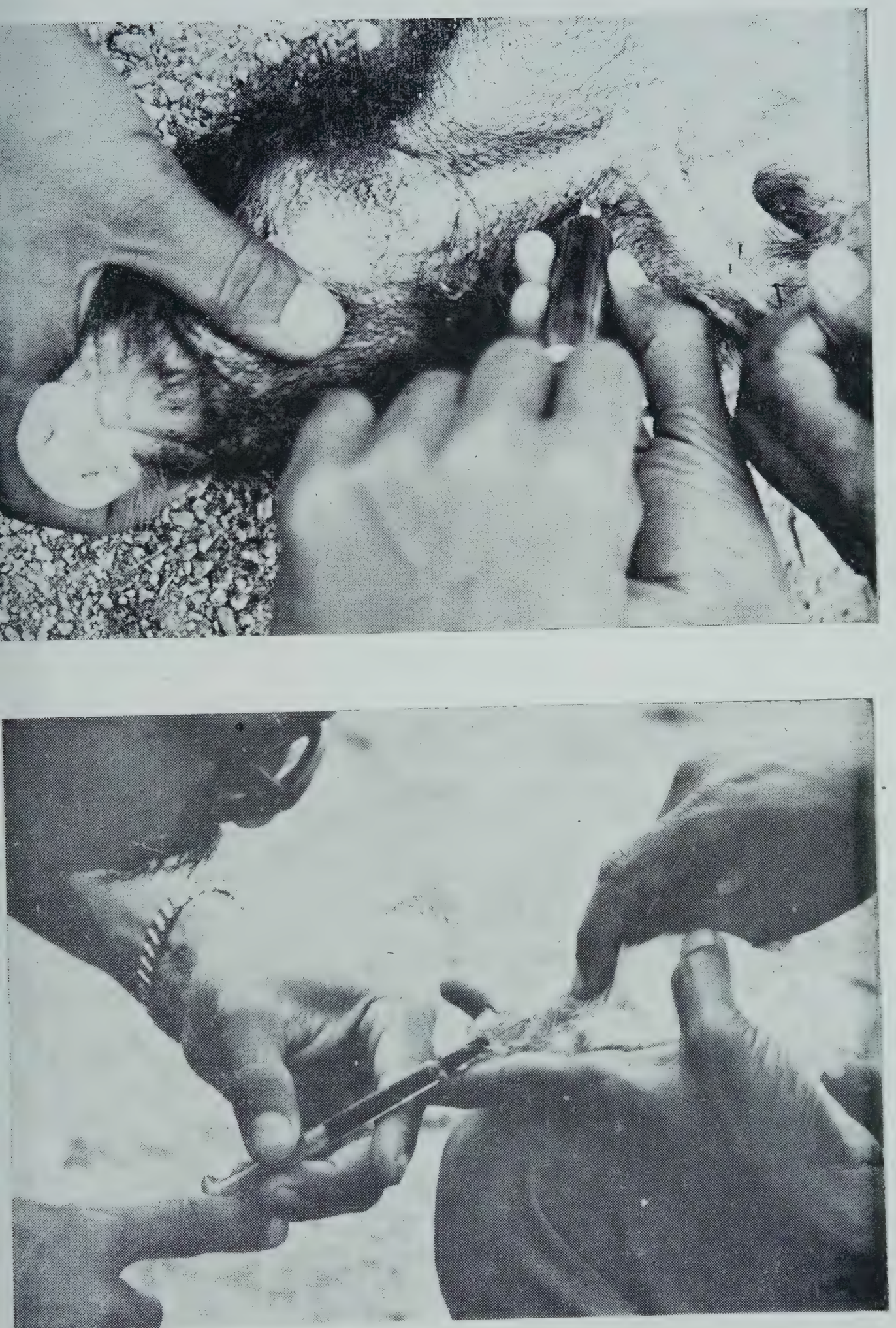
Though the earlier studies did not indicate the involvement of birds, the studies conducted later suggest the involvement of Ardeid birds at least in some areas. The possible role of ducks is yet to be determined with more evidence either by virus isolation or by serological studies.

In a total of 146 bird sera, collected from Asansol (WB) and Dhanbad (Bihar), N antibodies against JE virus were found in 9 out of 34 little egrets, one of 15 cattle egrets, and one of 13 paddy birds. One of the four crow sera and three of the 65 duck sera also showed antibodies against JE virus.

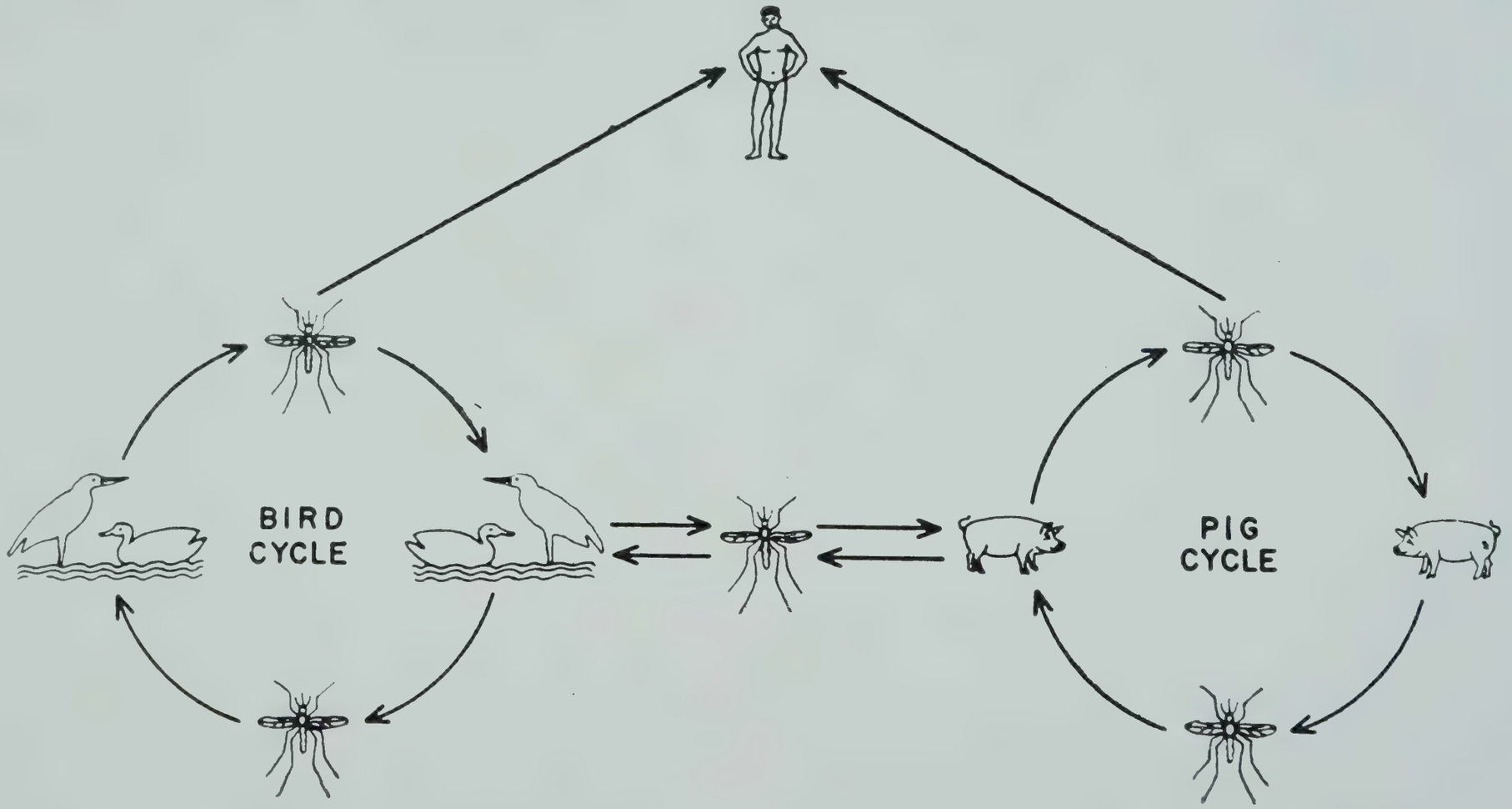
Pigs: Since the beginning of the investigations on the JE natural cycle, pigs have been incriminated as the major vertebrate host for JE virus in Malaysia, Singapore, Taiwan, Korea and Japan. In India, pigs have been implicated in areas around Vellore in Tamil Nadu on the basis of a study conducted to search for extra-human vertebrate reservoirs. About 30 per cent of the pigs were found immune. On experimental infection, pigs developed adequate titres of virus to infect mosquitoes. Subsequently, serological surveys of pigs have been carried out in different parts of India. The percentage of antibody positives in pigs against JE varied from 1.2 to 44 per cent in different parts of the country.

It may be noted that in the Pune region of Maharashtra, JE activity is low. This is also supported by the extremely low incidence of antibodies to JE virus in pigs. In Tamil Nadu which is a JE-endemic area, the proportion of pigs possessing antibodies for JE virus was high (44 per cent).

The percentage of pigs immune to JE in East and South-East Asian countries is much higher than in India, sometimes as high as 100 per cent. Also, in



Blood being collected from a pig (above) and a bird (below), the animals that play an important role in the natural cycle of Japanese encephalitis virus in India



PROBABLE NATURAL CYCLE OF JAPANESE ENCEPHALITIS VIRUS IN INDIA.

India there is a much lower ratio of pigs to the human population than found in other Asian countries with pork-eating populations. This may be the reason for the relatively lower incidence of JE in India. The relative role of pigs vis-a-vis Ardeid birds and other birds remains to be determined. In studies conducted in Bankura, a positive and significant correlation was found between the number of pigs in a given area and the total number of infected cases. A significantly large number of cases also came from scheduled castes and scheduled tribes who lived at the bottom of the socio-economic ladder and were the only communities who reared pigs and lived in close association with them.

Bovines: In a serological survey carried out at Vellore, 57 per cent of buffaloes and 27 per cent of cattle were found to possess N antibodies. Fifty per cent of buffalo sera and 26 per cent of cattle sera collected during and immediately after the 1973 outbreak in Bankura had HI antibodies. N antibodies were detected in 60 per cent of these HI-positive sera. It is evident that cattle and buffaloes become infected with JE virus by the bite of infected mosquitoes. However, two young buffaloes inoculated with large doses of JE virus failed to develop viraemia indicating that these animals might not be involved in the maintenance of the virus.

Bovines are unlikely to play a role in the maintenance and spread of JE. India has an extremely large cattle population. Studies in south India have shown that the vector mosquito for JE, *C. tritaeniorhynchus*, has predilection for cattle. Under the circumstances, there should have been explosive annual outbreaks of JE in India. However, the incidence of human cases of JE in India is much lower than that in Japan and in most countries of East and South-East Asia.

The lower incidence of JE in India may be explained by the predilection of the vector mosquitoes

for cattle. A large proportion of infected mosquitoes feed on cattle which are "dead-ends" for the transmission. Thus the high cattle-pig ratio dampens JE virus activity in nature.

Man and other vertebrates: Isolation of JE virus from human blood is rare and it seems that man is not a suitable host to infect mosquitoes. On the other hand, *C. tritaeniorhynchus*, the major vector though mainly a zoophilic mosquito, may bite man in the absence of the usual host or when its population is high.

Among the animals' sera collected at Vellore and tested for JE neutralising activity, none of the frogs snakes and lizards showed neutralizing antibodies. Of the 20 monkeys tested, none had antibody nor did any of the 156 rodents or the 25 bats. However, viraemia at low levels has been demonstrated in bats *(R. leschenaulti)* for 8-9 days after experimental infection.

The available evidence implies Ardeid bird-mosquito-Ardeid bird and pig-mosquito-pig cycles in nature. Man appears to be only an incidental "dead-end" host (Diagram). The Ardeid bird-mosquito-Ardeid bird cycle may be more stable, persistent and enzootic. The pig-mosquito-pig cycle appears to be temporary and epizootic. The epidemic in man appears to follow pig epizootics. There might be several other vertebrates and mosquitoes involved on the periphery of these cycles as complementary factors. These need careful assessment.

CONTROL MEASURES AGAINST JE VIRUS

In the natural cycle of JE virus, man appears to be a "dead-end". Man to man transmission has not so far been recorded. Among vertebrate hosts, pigs circulate virus in titres high enough to infect large numbers of mosquitoes. The pigs, however, do not show any signs of illness, except abortion as reported in Japan. In India, however, there is no evidence of abortions

in pigs due to JE virus. The role of cattle and buffaloes needs to be considered more as attractants to mosquitoes. Where people share their lodgings with their cattle, mosquitoes attracted to the animals might occasionally bite humans. The role of cattle in the epidemiology of JE would be to support large numbers of mosquitoes by providing them with adequate blood meal. Horses, on the other hand, develop encephalitis and succumb to the infection. In areas where the population of horses is in sufficient number, death of horses may herald JE outbreaks in humans. The role of other equines, such as mules and donkeys, is not known in India.

The control measures, therefore, need to be directed towards: (i) control of the mosquito vectors; (ii) prevention of mosquitoes from biting humans; (iii) vaccination of the humans; and (iv) measures against reservoirs.

Vector control

In India several species of mosquitoes appear to be involved in the ecology of JE virus. It also seems likely that in different parts of the country different mosquitoes may play a major part in the transmission of the virus to humans. It is, therefore, difficult to recommend control measures during the inter-epidemic period.

Considering the vast areas serving as breeding places for the mosquitoes and the inadequate information on the breeding habitats in different areas, larvicidal measures do not appear to be practicable at present. Aspects of vector control which are of the nature of exploratory research are discussed later.

The following practical measures are recommended for implementation particularly by the State and District health authorities in the known "high risk" areas of JE:

1. Information on the prevalence and density of the mosquitoes in the respective areas is to be obtained.

2. Studies on insecticide susceptibility of the known and potential vectors of JE should be undertaken and the information made available.

3. Surveillance of adult mosquito populations to be carried out throughout the year. Any sharp increase in the density of the known or potential vectors of JE should be recognized and followed up by alerting the medical component of the public health authorities, so that they are ready to recognize immediately patients of JE.

4. Sharp increase in the vector density should also be followed by prompt and adequate mosquito control measures using appropriate insecticides. Personnel trained in entomology, working on malaria surveillance programme, are available at the district level in most of the states. These personnel may be entrusted with the surveillance of mosquitoes acting as vectors for the JE virus.

5. Spraying of an appropriate insecticide should be carried out in resting places of the mosquitoes, *viz.*, inside houses, cattle sheds, pigsties, chicken coops *etc.* Spraying of the vegetation surrounding the affected villages should also be carried out as this is an important resting habitat for the mosquitoes.

Prevention of mosquito bite

1. Motivation and education of the population at risk through pictorial posters, radio and other mass media so as to avoid exposure to mosquitoes.

2. Improvisation and provision of some cheap feasible type of mosquito net to be used in houses.

3. Provision of suitable mosquito repellents at subsidised rates with proper instructions regarding their application to the population at risk.

Vaccine

Vaccination of the population at risk is the method of choice for the prevention of JE. In Japan, a vaccine obtained by inactivating the virus with formalin has been used. The virus for the vaccine is obtained from infected mouse brain. The vaccine is normally kept at refrigerator temperature and its shelf-life is usually one year. To increase the shelf-life, it can be lyophilised. Details of vaccination are given in Appendix IV.

Long-term studies in Japan, based upon the incidence in the vaccinated and unvaccinated populations showed that the vaccine was truly effective in reducing the incidence among the vaccinated population. However, the vaccine is not 100 per cent effective. There have been confirmed cases of the disease occurring among vaccinated individuals. Immunity produced by the vaccine is relatively short lived.

Protection of reservoirs

Pigs seem to be the most important amplifying hosts of JE virus. Building of piggeries away from human dwellings and making them mosquito-proof would be desirable. Enforcement measures for making the newly established commercial piggeries mosquito-proof should be ensured. Spraying of the piggeries and the mixed dwellings with suitable insecticides whenever there is an alarming rise in vector species, should be carried out promptly. Vaccination of pigs in Japan has given encouraging results. In India, there is a common practice, particularly among the lower socio-economic groups to rear pigs as scavengers and also for consumption. Therefore, implementation of such measures could be considered only in large commercial piggeries.

The Animal Husbandry Departments of the states should be asked to report any unusual rise in death rate amongst horses and other equines due to encephalitis and increase in abortion rates among pigs.

SUGGESTIONS FOR FUTURE RESEARCH

Retrospective studies on the vast collection of sera/data already available at the Institute will be undertaken. Before considering the future areas of research, the following features peculiar to India need to be considered.

Unlike in most other countries of South-East Asia, the closely related West Nile virus is also prevalent in many parts of India. Occurrence of both these viruses in the same area has several implications: (i) It becomes difficult to assess the specific nature of antibodies both in human as well as extra human hosts; (ii) presence of antibodies to WN virus alone or in combination with some other flaviviruses, such as dengue, may possibly assist in providing certain protection against severe disease with JE virus; and (iii) active and concurrent circulation of these two viruses may lead to an emergence of mutant strains.

Research areas

1. West Nile virus apparently infects a wider variety of mammals and birds as compared to JE virus. This creates confusion in assessment of the exact serological status of individuals particularly in areas where both these viruses are prevalent. Therefore, the following definitive studies remain to be carried out:

(i) Assessment of clinical to sub-clinical ratio for JE.

(ii) Evaluation of serological response to JE vaccine (currently obtained from Japan) among the

populations with previous antibodies to flaviviruses and those without antibodies.

(iii) Determination of specific identity and the role of mammalian and avian reservoirs/amplifiers in the natural history of JE virus.

Rapid method for specific identification of the virus isolates has already been developed at the NIV (see page 19). Attempts need to be directed to develop antigens which would specifically detect antibodies only to JE virus. Specific soluble antigens, ELISA test or thin layer immunoassay and such other techniques should be tried.

2. (i) Research to determine the incidence and severity of the disease due to JE virus among the population with antibodies to WN and dengue viruses and those lacking such antibodies.

(ii) Experiments in animals to determine the effect of sequential immunization.

3. Studies on genetic, biological and antigenic properties of different strains of JE virus isolated from patients/mosquitoes during different epidemics. Studies on the structural polypeptides of JE and WN viruses and the genetic homology of these viruses have already been initiated at the NIV.

4. Epidemiological research:

(i) Socio-economic and environmental factors associated with the incidence of the disease.

(ii) Natural cycle of JE virus in selected areas.

5. Immunological and pathological studies:

(i) Nature of antibodies in different stages of illness.

(ii) Pathogenesis of JE virus infection in patients and experimental animals.

6. Research on control measures:

(i) Development of vaccine by using indigenous strains.

(ii) Development of candidates for attenuated vaccine.

7. Research on vector control:

(i) In a country like India, where extensive breeding places for mosquitoes exist in the form of rice fields and innumerable stagnant water ponds, tanks, pools and marshes, larval control by existing methods is a difficult proposition. Therefore,research to find alternative control measures to be supplemented with conventional measures for achieving long-term control, is needed. Research on biological control including studies on larvivorous fish, parasitic nematodes, protozoans, fungi and bacteria should be carried out. Potential of certain insect viruses for this purpose also needs to be explored. The possibility of intermittent irrigation in certain areas so that rice fields are flooded for a limited period during which complete development of larva to adult is not possible, is worth consideration.

(ii) Standardisation of the use of chemical insecticides for the abatement of adult population is necessary. Research on the susceptibility status of mosquito vectors to various insecticides is necessary so that the control of adults by the use of ULV spraying can be achieved.

8. Other aspects of vector research:

(i) Several species of mosquitoes are suspected to be involved in the ecology of JE virus in India. Among these only *Culex tritaeniorhynchus* is implicated to be the most important vector in south India. There is strong evidence that *Culex bitaeniorhynchus* acts as a vector in some areas but more isolations from wild-

caught mosquitoes are required before this can be confirmed. Other suspected species include six culicines, *viz.*, *C. vishnui*, *C. pseudovishnui*, *C. gelidus*, *C. whitmorei*, *C. epidesmus* and *C. fatigans* and three anophelines, *viz.*, *A. hyrcanus*, *A. barbitostris* and *A. subpictus*. Evidence regarding their role is at present inadequate and further research, both in the field and in the laboratory, is necessary so that the target species for control operations may be more clearly defined.

(ii) At present, knowledge on the ecology of several of the suspected vector species is scanty. Such studies, therefore, need to be carried out expeditiously.

(iii) A great deal of information has to be collected with regard to taxonomy, distribution and bionomics of culicine mosquitoes, particularly the genus *Culex* in India.

APPENDIX I

COLLECTION AND TRANSPORT OF SPECIMENS FROM PATIENTS

1. Specimens for virus isolations*

CSF:

Virus isolation should be attempted from blood, CSF and post-mortem brain tissue. Isolation of JE virus from the blood collected during the acute phase of illness is rare. Virus has been isolated from CSF (1-2 ml) collected in the acute phase of illness, *i.e.* day 0 to day 5 of onset. The earlier the specimens are collected the greater the chances of virus isolation.

Serum:

Sera for virus isolation should be collected as early in the illness as possible. Four to five ml of blood (to yield at least 2 ml of serum) should be collected. The blood should be kept at room temperature for about 15 minutes to enable it to clot. The clot is detached from the side of the tube by tapping the tube on the palm or with the aid of a sterile applicator stick. It is then placed in the refrigerator at 4°C (do not place in the freezer compartment) to allow the clot to retract and ensure maximum yield

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* It is essential that strict aseptic precautions are observed in the collection of specimens. All equipment used should be sterile and the specimens must be collected in sterile containers.

of serum. The serum is then separated from the clot and transferred into a sterile screw-cap container. If screw-capped containers are not available, the specimens may be placed in moderately thick walled rubber stoppered test tubes.

The containers should be sealed with adhesive tape. Adhesive tape should also be used for labelling. All labels should be clearly written and the labelling should be done in pencil. Ink should not be used as it runs on contact with water.

Brain tissue:

Brain tissue obtained from patients dying during the first two weeks of illness is the best source for the isolation of virus and the chances of isolation are highest from cases dying early. If an autopsy can be performed, small pieces of brain tissue should be obtained from different parts of the brain - cerebral cortex, cerebellum, basal nuclei and brain stem. If a full post-mortem is not possible, small pieces of cerebral tissue may be obtained with the aid of a trephine. If even this procedure is not permitted, a small piece of brain tissue can be obtained by biopsy using a Vim-Silverman needle inserted via the nose through the cribriform plate of the ethmoid bone.

The tissue(s) thus obtained should be immersed in 2 ml of transport medium (see Appendix II). If the reagents for the preparation of transport medium are not available, glycerol-saline may be used (see Appendix II). Alternatively, nutrient broth medium can be used but it has the disadvantage that contaminating bacteria may grow in the medium. The container used should be of moderately thick glass and should preferably be screw-capped.

Storage and transport of specimens for virus isolation:

The specimens should be stored and transported on solid CO_2 (dry ice; temperature - 79°C) or in liquid

nitrogen (temperature - 196°C). If facilities are available these are the methods of choice. However, for this purpose, the specimens should be sealed (flame-sealed) in ampoules made of good glass, care being taken to ensure that the specimens are not heated with the flame.

Sealed ampoules are necessary because dry ice CO_2 causes pH changes in the specimens resulting in inactivation of the virus and with liquid nitrogen, sealing prevents the liquid nitrogen from seeping into the container. Should this happen, the change from liquid to gas state and consequent expansion on removal from the liquid nitrogen refrigerator would cause the container to explosively disintegrate.

Specimens sent on dry ice should be properly packed and placed in a metal thermos or thermocole container filled with dry ice. The dry ice lasts for approximately 24 to 36 hours depending on the size of the container; hence it should be air-freighted to the National Institute of Virology (NIV), Pune, with prior telegraphic or telephonic intimation.

In order to send specimens in liquid nitrogen, a special liquid nitrogen refrigerator is required. The liquid nitrogen lasts between a week and ten days, if the refrigerator is well filled with liquid nitrogen, depending on the environmental temperature conditions. The specimens can thus be transported either by rail through a courier or air-freighted to the NIV, Pune.

In most instances, such facilities will not be available. Specimens should be placed in a refrigerator at 4°C as soon as possible after collection. The specimens should not be freezed. They should be despatched at the earliest possible opportunity in a large thermos or in an ice-box, to the NIV, Pune, on wet ice. They can either be air-freighted or sent by rail through a special courier. The courier should drain the water and replenish ice as and when required, during the journey.

2. Specimens for histopathology

Additional specimens of brain tissue in 10 per cent formalin should be sent to the NIV, Pune, for histopathological examination.

3. Serum samples for serological examination

Paired serum samples:

Acute-convalescent paired serum samples are very important in the diagnosis of Japanese encephalitis for the demonstration of significant rise in the titre of antibodies to Japanese encephalitis virus. The first sample should be obtained when the patient is first seen and the second sample two to three weeks later. About 4 to 5 ml of blood should be collected so that at least 2 ml of serum is obtained. The sera should be separated from the clot as described above and stored at 4°C in a refrigerator till they are despatched. Without freezing the sera it should be ensured that they are despatched to the NIV, Pune,within one to two weeks of collection. Sera should be despatched on ice, in an ice-box or thermos flask either by rail with a courier or by air freight.

Single convalescent-phase sera:

The patient is often first seen in the "convalescent-phase" of the illness, *i.e.* after ten days from onset. Sera collected in this phase are also useful as a presumptive diagnosis can often be made on the basis of antibody titres. The sera are collected, processed, stored and despatched as described above for paired serum samples.

4. Information required to accompany the specimens

The following information should always be given on the request form accompanying the specimens:

Full name and father's name of the patient,

Full address,

Age and sex,

Main clinical findings including clinicopathological findings,

Date of onset of symptoms,

Date when specimen was taken,

Name and address of the clinician.

APPENDIX II

PREPARATION OF TRANSPORT MEDIUM

Hank's balanced salt solutions

Solution A (Stock)

NaCl	40.0 g
KCl	2.0 g
$MgSO_4.7H_2O$	0.5 g
$MgCl_2.6H_2O$	0.5 g

Dissolve in 200 ml deionized H_2O. Dissolve 0.7 g $CaCl_2$ in 30 ml deionized H_2O. Mix and make up to 250 ml with deionized H_2O. Add 0.5 ml chloroform. Store at 4°C (stable for atleast one year).

Solution B (Stock)

$Na_2HPO_4.12H_2O$	0.76 g
KH_2PO_4	0.30 g
Dextrose	5.60 g

Dissolve in 200 ml deionized H_2O. Make up to 250 ml with deionized H_2O. Add 0.5 ml chloroform. Store at 4°C (stable for at least one year).

Working solution

Solution A	50 ml
Solution B	50 ml
H_2O	870 ml
0.4 per cent phenol red	5 ml

Distribute into bottles in desired volumes. Autoclave at 10 lb/in^2 for 15 minutes. Before use, adjust

pH as desired (approximately pH 7.2) with 4.4 per cent solution of $NaHCO_3$.

Bovine albumin solution

Prepare stock 10 per cent solution by dissolving 10 g bovine albumin, fraction V in 100 ml deionized water and sterilizing by Seitz filtration. Store at 4°C. This solution is used in transport medium and as an additive for stabilizing virus suspensions.

Penicillin-streptomycin mixture

Dissolve one million units crystalline penicillin G and 1 g streptomycin sulfate in 100 ml sterile Hank's BSS, distribute into 5 ml amounts and store at -20°C. For use, add 1 ml of this stock solution to 100 ml medium to give a final concentration of 100 units penicillin and 100 µg streptomycin. In addition mycostatin may be used.

Transport medium

This is a balanced salt solution containing added protein which helps to stabilize labile viruses. It may be prepared as follows:

Hank's BSS	87 ml
10 per cent albumin solution	10 ml
Sodium bicarbonate solution	2 ml
P-S mixture	1 ml

Distribute into screw-capped containers and store (for not more than one month) at 4°C.

Preparation of 50 per cent glycerol in phosphate-buffered saline, pH 7.5

Phosphate-buffered saline solution, pH 7.5 (PBS)

NaCl	8.00 g
KCl	0.20 g

KH_2PO_4	0.12 g
Na_2HPO_4 (anhydrous)	0.91 g
Double distilled water	1000 ml

This solution may be sterilized by autoclaving at 18 lb pressure for 35 minutes.

Add equal quantities of PBS and glycerol. The glycerol must be of analytical reagent grade and neutral. Prior to being added to PBS, it should be autoclaved at 10 lb/in^2 for ten minutes.

APPENDIX III

COLLECTION AND TRANSPORTATION OF MOSQUITOES

In an epidemic situation, it is desirable to collect mosquitoes from the affected houses and localities so that they may be processed for virus isolation. This will not only give an indication of the species acting as vector but also provide some information on the mosquito fauna of the area. Mosquitoes can be collected by the following standard methods.

Resting collection: Mosquitoes resting in houses, cattlesheds, pigsties and their surroundings may be collected using an aspirator (suction tube) and a torch-light.

Biting collection: Mosquitoes which are attracted to animal or human baits may be collected during dusk using the aspirator and torch-light.

Baited traps: Various types of traps depending upon the type of host to be used as a bait can be employed. The mosquitoes trapped on these baits,which are generally held overnight,can be collected by using an aspirator.

Light traps: Under certain situations, light traps may give good results.

The mosquitoes collected by any of the above methods should be held alive in Barraud cages for at least 24 hours before they are identified. If the collection locality is not far from the laboratory or transportation can be done within a day or two, they may be transported alive in Barraud cages. For such transportation it is necessary to provide raisins

soaked in water or a cotton pledget soaked in 10 per cent glucose solution inside the Barraud cage. To prevent desiccation, the cages should be wrapped in moist cloth. The attendant accompanying them should see that the cloth remains adequately moist but never dripping, throughout the journey.

In the laboratory, the mosquitoes are anaesthetized, identified under a stereoscopic microscope, pooled according to species and locality and either directly processed for virus isolation or if immediate processing cannot be done, the mosquito pools may be stored at -70°C or in liquid nitrogen refrigerator. If the collection locality is far from the laboratory and immediate transportation is not possible, mosquitoes may be identified and pooled in the field. These pools may be stored in liquid nitrogen refrigerators or on dry ice for transportation to the laboratory. If facilities for liquid nitrogen or dry ice storage are not available in the field, transport medium may be used to store the mosquito pools. It is, however, necessary that such pools must be constantly kept in the refrigerator or transported on ice.

APPENDIX IV

DIRECTIONS FOR USE OF KILLED JAPANESE ENCEPHALITIS VACCINE PREPARED FROM MOUSE BRAIN

Administration and dosage

For primary immunization, two doses of the vaccine, each of 1 ml (0.5 ml for children under 3 years of age), are administered subcutaneously. The interval between the two doses is 7 to 14 days.

For the booster immunization, a third dose of 1 ml (0.5 ml for children under 3 years of age) should be administered subcutaneously a few months to one year later. A further booster dose should be administered subcutaneously after 3 years. This may be all that is necessary for individuals living in the endemic zone. Those in the non-endemic zone may need further periodical vaccine boosters once every four years.

The vaccine is best administered in the inter-epidemic period because of the two injections and the time lapse of one month after the second injection, which is necessary for good protection. The vaccination programme should, therefore,be completed at least one month before an anticipated outbreak or before the epidemic season.

Reactions

Sometimes there may be redness, swelling or pain at the site of injection, but these disappear within a few days. As a rule, systemic reactions are rather mild. In some cases, however, headache, chills, fever

or malaise may occur; these symptoms do not require any medical treatment.

Contraindications

Vaccination is not advisable for persons suffering from ill health, persons with allergic diathesis or history of convulsions, and pregnant women unless they are at risk in the face of an outbreak. The vaccine has been administered to pregnant women in some countries and no adverse reactions have been noted.

Whom to vaccinate

The decision on whom to vaccinate will depend on local epidemiological factors and logistic considerations.

Age groups to be covered should be decided areawise, based on epidemiological findings, priorities established and the availability of the vaccine.

Infants under one year of age need not be vaccinated. However, as a precautionary measure, they should be protected from mosquito bites by the use of mosquito nets.

The size of the population at risk should be determined. Only an adequate coverage of 80 to 90 per cent of the population at risk will lower the morbidity rate. Otherwise, cases would continue to occur.

Storage and transport

The liquid vaccine has a rather short period of potency from the date of manufacture. The expiry date is indicated on the vial or ampoule. It must be kept at all times at a temperature between 4°C and 10°C. It must not be frozen. Because of these considerations, it is preferable to use the freeze-dried vaccine in warm climates.

The freeze-dried vaccine should also be kept at a temperature below 10°C. It can be stored at -20°C for a period of 3 to 4 years.

During transportation, the vaccine, whether freeze-dried or liquid, should be transported on wet ice in an ice-box. The freeze-dried vaccine can, however, be kept without refrigeration for a period of approximately one week.

APPENDIX V

OUTBREAKS OF JE IN 1978

From the last quarter of 1977 through 1979, the NIV investigated a number of outbreaks of encephalitis. These occurred in widespread geographic areas as detailed in Table 1. The important species of mosquitoes collected in various parts of India during or subsequent to the JE epidemics, are shown in Table II.

Natural history of JE virus

As a part of the arbovirus programme, the NIV has been studying the natural history of some viruses of public health importance. Studies on JE virus to understand the role of different animals as possible reservoirs and of mosquito vectors of the virus have been continued.

Serological tests on sera from birds and animals

Sera collected from water-inhabiting and water-frequenting birds from Krishna and West Godavari districts of Andhra Pradesh were tested. Neutralizing (N) antibodies against JE virus were detected in 38.5 per cent (110 out of 286) of *Ardeola grayii* (pond heron) and 37.1 per cent (85 out of 229) of *Bubulcus ibis* ((cattle egret). Only 3 per cent (10 out of 338) of the sera from ten other species of birds contained N antibodies against JE virus. In view of the serological cross-reactions among the flaviviruses (group B arboviruses), 190 sera from Ardeid birds in which JE antibodies were demonstrated were also tested for antibodies to a closely related virus, *viz.*, West Nile (WN) virus. N antibodies to WN virus were found in 141 sera. The specificity as to which virus is mainly

involved remains to be worked out. However, these findings indicated the involvement of pond herons and cattle egrets in the natural cycle of JE/WN viruses in southern India. Sera from birds and animals collected from other areas remain to be tested.

Transmission studies with JE virus

Attempts were continued to transmit JE virus through *Anopheles "hyrcanus"*. The results show that the species of mosquitoes collected in Maharashtra did not transmit the virus or at least, the rate of transmission would be too low to be of any epidemiological significance.

Transmission with *Culex bitaeniorhynchus*

Culex bitaeniorhynchus which has been found to be an efficient mosquito vector could transmit the virus up to 40 days after infection in the laboratory.

Colonization of insects

For transmission experiments with JE virus, attempts are being made to colonize suspected or potential vectors of JE, *viz.*, *Anopheles subpictus*, *A. barbirostris*, *A. "hyrcanus"*, *Culex fuscocephalus* and *C. whitmorei*.

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